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(54) Title: FABRICATION AND METHOD OF USE OF CODED PARTICLES IN THE FIELD OF COMBINATORIAL CHEMISTRY

(57) Abstract

A method for screening to identify a compound of interest from a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available in a database to identify the sequence of reactions experienced by substantially each support particle, the method comprising the steps of: (a) dividing the compound library into two sets there being a statistically satisfactory representation of all compounds in the library within each set; (b) sub–dividing a first one of the two sets into a number of sub–sets; (c) testing for a chemical activity of interest in any one of the said sub–sets and identifying the one or more sub–sets showing the activity of interest and reading the machine readable codes of the support particles in that or those sub–set(s); (d) checking a database of the tracking data, or data established from the tracking data, to identify machine readable codes of any other support particles which have been through the same steps and therefore, have the same chemical structure of compound on the support particle; and (e) reading the machine readable codes of the support particles in the second set of particles to locate the one or more support particles having the corresponding machine readable code(s) and retrieving that or those support particles and subjecting it or them to further screening tests to identify the compound of interest.

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FABRICATION AND METHOD OF USE OF CODED PARTICLES IN THE FIELD OF COMBINATORIAL CHEMISRTY

Field of the Invention

The present invention relates to coded Combinatorial Chemistry particles. It is particularly applicable to improved methodology for reading and using the coded particles.

Background to the Invention

A method of fabricating and using coded particles in the area of combinatorial chemistry library synthesis is described in GB 2306484 B which describes the fabrication of coded particles of dimensions typically in the order of 100μm length and width and typically in the order of 20μm thickness. Additionally, the particles are encapsulated by, coated with, or otherwise attached to, a similar volume of polymeric resin material which acts as a substrate for the chemical compound growth during the combinatorial chemistry process. Each particle carries a unique code, thus allowing individual particles to be tracked throughout the combinatorial compound library synthesis, an essential requirement in allowing the compound synthesis sequence for compounds which exhibit the desired biological, pharmacological or chemical activity to be determined and reproduced. A more detailed description of such a synthesis is given in GB 2306484 B, the entire contents of which are hereby imported by reference. It is intended that this earlier disclosure should form an integral part of this present application.

A method of manufacture of the coded particles is described in GB 2306484 B. For convenience, a brief summary is provided as background information here. The manufacture is based upon the technology of micromachining, a technology originally developed from the microelectronics industry and which uses similar processes of deposition and etching to those used to fabricate microelectronic integrated circuits. The fundamentals of the fabrication of the particles is described by Kaye et. al. in the Journal of Aerosol Science Vol. 23 [1992, Supplement 1, 201-204] and typically involves:-

(i) the design of the required particle geometry (or geometries) using computer aided design (CAD) tools;

(ii) the manufacture of appropriate photolithographic masks which delineate both the particle outlines and the required coding marks within the particle outlines;

(iii) the growth by vapour deposition onto a polished silicon wafer (typically 3, 4 or 8 inches in diameter) of a thin layer of material such as aluminium, this to become a sacrificial bonding layer (see below);

(iv) the growth by vapour deposition or similar process, onto this sacrificial layer of a further layer of, for example, silicon or silicon dioxide from which the particles will be ultimately be formed;

(v) the coating of this layer with a photosensitive polymer resist (photoresist) which, upon ultraviolet exposure through the photolithographic mask, defines the particle shapes and the locations of coding marks;

(vi) the removal of exposed photoresist thus revealing areas of the underlying layer of silicon or silicon dioxide; and

(vii) finally the creation of the particles with their associated code marks within

this layer by etching away the revealed areas of the layer between the particle outlines and within the areas designated as code marks. The particles may then be freed from the wafer substrate by dissolution of the sacrificial layer which underlies the particles. The sacrificial layer is typically made of aluminium if the particles are of silicon dioxide, and of silicon dioxide if the particles are of silicon.

In order to render the particles suitable for use in combinatorial chemistry, an additional processing stage is required before the particles are freed from the wafer substrate. In this additional stage, each coded particle receives its quantity of polymer support material attached to the particle in the form of a chemically attached surface layer, by mechanical linkage, or by some other method of attachment as described in GB 2306484 B. The microscopic code on the particles may be interrogated and read using contemporary microscope-based image processing systems. By way of example, a code containing just twenty binary sites (pits, holes, or similar features) would allow a million particles to be uniquely numbered from 1 to 1,000,000.

The accepted method of manufacture of the coded particles as described in GB 2306484 B is limited in the maximum thickness which the particles can assume. Because the particles are created from a deposited layer, this maximum thickness corresponds to the layer depth above which material stresses can result in particle fracture when the particles are freed from the host substrate wafer. Typically this thickness if of the order of 10-20 micrometres. In some areas of combinatorial chemistry, it is valuable to produce more compound per particle than may be supported by a particle of this size. However, if the particle length and width are

increased to allow a greater loading of polymer resin (and hence a greater compound yield) whilst retaining the thickness at its maximum level, a thin wafer-like particle results which has poor mechanical robustness. Therefore an alternative approach to particle fabrication is required which allows larger particles to be produced without the risk of particle fracture. This need is addressed by the method described in the following section. The section also describes a method by which the codes on these larger particles may be efficiently read, and methods by which further advantage can be gained from the efficient process of code reading and particle tracking which is a feature of the machine-readable coded particles.

Summary of the Invention

According to a first aspect of the present invention there is provided a method for screening to identify a compound of interest from a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available in a database to identify the sequence of reactions experienced by substantially each support particle, the method comprising the steps of:

(a) dividing the compound library into two sets there being a statistically satisfactory representation of all compounds in the library within each set;

(b) sub-dividing a first one of the two sets into a number of sub-sets;

(c) testing for a chemical activity of interest in any one of the said sub-sets and identifying the one or more sub-sets showing the activity of interest and reading the machine readable codes of the support particles in that or those sub-set(s);

(d) checking a database of the tracking data, or data established from the tracking data, to identify machine readable codes of any other support particles which have been through the same steps and therefore, have the same chemical structure of compound on the support particle; and

(e) reading the machine readable codes of the support particles in the second set of particles to locate the one or more support particles having the corresponding machine readable code(s) and retrieving that or those support particles and subjecting it or them to further screening tests to identify the compound of interest.

Preferably, in the method prior to step (b) the compound bound to each support particle is released by relieving the compound linking it to the support particle to release each compound into a liquid medium .

In step (c) prior to reading the machine readable codes of the support particles they are preferably removed from a vessel in which they were tested for chemical activity and transferred to a reading station.

Advantageously the reading of the codes on the coded particles is carried out by firstly placing the particle to be read in a substantially flat, substantially horizontal vessel with a transparent bottom, the area of the bottom being at least double the total area of the particles to be measured; agitating the vessel to form a monolayer of particles on the bottom; and scanning the vessel with an imaging system whilst illuminating the particles.

Preferably the reading station is adapted to automatically read the machine readable codes of the support particles and to automatically identify the one or more support particles with machine readable codes in step (e) that correspond to the codes from the database identified in step (d).

Suitably the one or more corresponding support particles are automatically retrieved or pinpointed by a laser beam, or other means, from the second set of particles.

According to a second aspect of the present invention there is provided a system for use in the method of any preceding claim and which comprises at least two primary vessels each containing a respective one of the two sets of support

particles, a reading station to read the machine readable codes of the support particles; a database of the machine readable codes and tracking data; and means programmed to identify the machine readable codes of the corresponding support particles ("clones") to those in the sub set which has been found to have the chemical activity of interest.

The system suitably further has an automatic retrieval means to pinpoint by a laser beam or other means and/or to robotically retrieve the support particles ("clones") of interest.

According to a third aspect of the present invention there is provided a method for identifying a group of different but related chemical compounds from a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available in a database to identify the sequence of reactions experienced by substantially each support particle, the method comprising computer searching the database to determine each machine readable code corresponding to a compound structure within the group of compounds of interest.

Preferably retrieval of the support particles corresponding to the thus-identified machine readable codes is automated either by the relevant particles being high-lighted by designating means such as, a laser or other means and/or fully automatically retrieved by means of robotic automation.

According to a fourth aspect of the present invention there is provided a method of building and deconvoluting a combinatorial compound library comprising the steps of:

(a) providing a plurality or set of support particles each with a machine readable code and a data base for tracking data to enable the sequence of synthesis reactions experienced by substantially each support particle to be identified;

(b) suspending the support particles in a fluid;

(c) dividing the fluid containing the particles into a plurality of portions, reading and recording the machine readable codes during or after the division process in order to track the movement of specific particles into respective portions;

(d) subjecting respective portions to specific chemical reactions;

(e) recombining the respective portions; and

(f) repeating steps (c), (d) and (e) as necessary so as to create a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available to identify the sequence of reactions experienced by substantially each support particle, and wherein following a division of the particles the codes are read and analysed to determine whether any combinatorial permutation is significantly under represented.

Suitably any under-representation of any permutation of chemical combination is adjusted for by re-mixing all or some of the sub sets of particles and, optionally, re-reading the machine readable codes.

Description of the Drawings

The present invention will now be described, by way of example only, with reference to the accompanying drawings wherein:-

Figures 1A and 1B illustrate in perspective view coded particles onto which one and two polymer deposits respectively have been made;

Figure 2 illustrates plan and side views of a resin bead trapped within an aperture in a coded particle.

Description of Preferred Embodiments

The present invention will now be described by way of example only. This represents the best ways known to the applicants of putting the invention into practice, but they are not the only ways that this can be achieved.

Improvements to Particle Fabrication

In the method described in GB 2306484 B the particles are formed by etching a layer of material which had previously been deposited onto a flat substrate, such as a silicon wafer. In contrast, the improved method described herein involves the etching of the silicon wafer (or other suitable solid) itself to form the particles. In this respect the method is comparable to that described in UK patent application 9803182.6 where thinned silicon wafers may be anisotropically etched using a potassium hydroxide etchant to produce particle boundaries and pyramidal-shaped code holes which follow crystal planes. The text of GB 9803182.6 is hereby imported by reference. The disclosures and methods described therein are intended to form an integral part of the present application.

However, one disadvantage of this earlier approach is that, because the holes are pyramidal, their size (in order to penetrate through the particle) is governed by the wafer thickness from which the particle is formed, with thicker wafers requiring larger and more widely spaced code-holes. For thicker wafers therefore, this can limit the size of the code number which the particle can support.

In the current invention the particle fabrication method is based upon a 'double-sided polished' silicon wafer from which the particles are etched. Such wafers are normally of the order of 300 micrometres thick, though thinner wafers, down to tens of micrometres thickness are now commercially available (for example from

Compart Technology Ltd, Peterborough, UK). The double-sided polished silicon wafer is first anodic-bonded to a substrate material such of glass or by other means to other rigid material such as an organic solid. In the preferred embodiment a Pyrex glass wafer is used.

However, any suitable substrate may be used which has sufficient mechanical strength and chemical stability to withstand the rest of the process. This includes certain rigid polymers and wax blocks, such materials being selected by the materials specialist.

The upper surface of the silicon wafer is then coated with an appropriate photoresist material which is subsequently exposed to ultraviolet radiation through a photolithographic mask which defines the size and shape of the particles and their respective code marks. The particle size thus defined will be selected in accord with the wafer thickness, such that the length and width dimensions of the particle are preferably several times the particle thickness so as to ensure a high probability that a free particle will lie horizontally, thus facilitating code reading. For a 300 micrometre thick wafer therefore, particles of the order of 700 micrometres square would be appropriate. Thinner wafers would allow commensurately smaller cross-sectional dimensions. Unexposed areas of photoresist are removed by an appropriate solvent to expose unwanted areas of the silicon surface. These unwanted areas are the inter-particle material plus the sites in the particle defining the code marks. A plasma etch process may then be used to etch the exposed material of the silicon wafer through the entire wafer thickness but without any etching of the underlying bonded glass wafer: the etching stops at this interface. Both the particles and their code 'holes' are thus formed in the same operation.

Plasma etching machines capable of etching through the entire depth of a wafer are relatively new tools for use in micromachining. They offer the unique benefit of allowing vertically-sided channels to be etched through the entire silicon thickness. This is in contrast to other etching processes which either 'undercut' the masked areas of silicon or are constrained to follow crystal planes (such as is the case with a potassium hydroxide wet etch). Such Plasma etching therefore allows the high precision fabrication of coded particles with the minimum inter-particle and inter-hole area wastage. The resulting etched glass-silicon composite wafers are then subjected to normal microengineering operations to remove photoresist masking layers and any contamination. Subsequent to this a silicon dioxide layer is deposited (typically 30nm) onto the surface of the etched particles in order to provide an optimal surface for the subsequent adhesion of polymer resin, as described below.

The coded silicon particles cannot themselves support compound growth during combinatorial chemistry synthesis. It is therefore necessary to affix to each particle, mechanically or chemically, a quantity of suitable polymer or other material to afford such a compound growth platform. In a preferred embodiment, a method of 'silk-screening' the polymer onto each particle is used. Silk-screening is an established technology used in the electronics industry for marking features on printed circuit boards. The screens may indeed be of silk or similar fibrous material, but may also be in the form of a thin metal sheet in which holes define the areas of desired polymer application. Such a mask may be used here. The mask overlays the particles (which are still attached to the glass substrate) such that the mask holes correspond to the areas on the coded particles to which polymer is to be applied. The polymer mix, prior to curing and consequent cross-linking, is applied though the mask using a screeding, painting, or rolling process. The silicon dioxide surface

may also be advantageously pre-treated with a silane compound designed to provide a 'coupling' functionality between the silicon dioxide and the polymer, hence promoting good adhesion. Also, the area of the particle surface to which polymer is to be applied may itself be etched by appropriate masking and plasma etching to leave a well or wells into which the polymer is forced, again promoting good adhesion between the particle and the polymer.

The use of a silk-screen mask in this way allows accurate simultaneous deposition of polymer to each particle on the wafer, possibly many thousands of particles in total. As indicated in Figure 1, each particle 1 would thus exhibit a code in the form of holes 2, and an area or several separate areas of polymer 3. The exact positions of code holes and polymer would be such as to ensure unobscured viewing of the code holes. Once the polymer has been deposited in this way, the temperature of the whole wafer assembly may be raised to an appropriate level to ensure cross-linking of the polymer takes place, whereupon the bond between the polymer and the particle becomes permanent. Finally the resulting polymer-patterned glass silicon composite wafer may be immersed in hydrofluoric acid in order to dissolve ('sacrifice') the glass substrate, thereby releasing the discrete silicon particles into the acid. The acid may be subsequently removed and the particles recovered for use.

In some cases the use of an encapsulating layer (for instance a wax-like compound) resistant to HF to protect the upper surface of the silicon-glass composite subsequent to polymer application and curing would be advantageous. This facility would serve both to protect the aforementioned silane and additionally provide for the use of a polymer other than polystyrene which may be desirable for chemical

compound synthesis reasons as such alternative polymers may not share the attribute of polystyrene of being unaffected by immersion in hydrofluoric acid, HF. The glass would then be dissolved by HF from below with the encapsulation protecting the upper surface layer from attack. Subsequent to HF processing the encapsulating layer would be dissolved or melted in a manner compatible with the polymer.

In an alternative method of linking the polymer to the coded particle, conventional polymer resin beads, currently widely used in combinatorial chemistry as solid phase supports for compound growth, may be used. This has the advantage that the chemistry of the beads is well characterised and would therefore be amenable to current synthesis practice. In a further preferred embodiment, resin beads are sieved through appropriate precision sieves so as to separate out a population having a narrow size distribution. For example, beads may be sieved to produce a monodisperse population of nominally 400 +/- 5 micrometres in size. The etching of the silicon wafer to produce the coded particles proceeds as before, but with the addition that a locating hole is etched in each particle into which a polymer resin bead will subsequently be positioned. As illustrated in figure 2, the particle 4 would exhibit code holes 5 plus a bead entrapment hole 6 which is of a size and form designed to rigidly hold the bead 7 in position whilst allowing the bead to act as a suitable substrate for compound synthesis.

A preferred method by which the beads are positioned in the entrapment holes is as follows. The beads are known to swell and contract when in contact with the various organic solvents used in combinatorial chemistry. It is essential that the bead and host coded silicon particle remain in contact throughout the combinatorial process.

The size of the entrapment hole is therefore chosen to be fractionally less than the smallest size which the bead is likely to assume during processing. This would normally occur when the bead is immersed in diethyl ether or similar reagent. The polymer beads may be linked to the silicon particles in the following way. When the silicon particles are etched but still attached to the glass substrate, the suitable population of resin beads is prepared by immersion in diethyl ether, causing them to contract to minimum dimensions. The collection of beads is then poured over the wafer surface and a roller or similar device used to compress the beads against the silicon particles. In areas where a bead becomes positioned over an entrapment hole, the bead will be forced into the hole. Repeated cycles of this process will ensure that the vast majority of the particles entrap a bead. Loose particles may then be flushed away and the wafer immersed in hydrofluoric acid as before to release the particles from the glass substrate. Each silicon particle will then contain a polymer resin bead exposed on upper and lower surfaces, allowing expansion of the bead without it being dislodged from the host silicon particle.

It is intended that this disclosure should encompass particles made from material other than silicon. Any suitable material can be used providing it will undergo the process steps described or their technical equivalent. For example, metals such as gold or compounds such as silicon dioxide could be substituted for silicon.

The invention also includes other methods of trapping a polymer resin bead such as those described in GB 9803182.6.

Reading the Codes

In the original patent (GB 2306484 B), the coded particles therein described were

small enough to be manipulated by suspending the particles in liquid and initiating suitable liquid flows. In this way, for example, a flow of particles could be made to pass through the sensing region of a code reading station (based on a digital imaging system) to allow the acquisition of images of the particles and subsequent reading of the codes. In the present invention where larger particles are used (in order to support a larger volume of polymer resin for compound growth), the use of liquid flows to move particles through a reading station is more difficult because of the negative buoyancy of the particles. An alternative method is therefore preferable.

In a presently preferred method, the collection of particles whose codes are to be read are poured onto the transparent surface of a flat horizontal vessel or similar container. Many thousands or many tens of thousands of particles may be involved. The particles may be dry or wetted with one of the liquid reagents used in combinatorial chemistry. The flat surface of the vessel is chosen to have an area several times that of the total surface area presented by the particles when lying flat, so that particle overlapping is minimised. The vessel is then placed onto a suitable mechanically driven stage which allows movement of the vessel in either an 'x – y' motion 'or x + rotation' motion. The vessel is then viewed from above by the stationary imaging system which is capable of acquiring still images of the particles. By moving the stage in an 'x – y' raster scan beneath the imaging system, it can be ensured that all the particles contained within the vessel are at some time brought within the field of view of the imaging system, and their code information recorded. Alternatively, an 'x + rotation' motion stage will allow the surface of the vessel to be scanned in either a spiral scan or a series of concentric circular scans. Again, at some time all particles will be brought within the field of view of the imaging system.

Illumination of the particles can be from below, in which case the code holes appear as bright spots against a dark background, or from above, in which case (because of the surface reflectivity of the silicon) the code holes appear as dark spots against a light background. Both forms of illumination can be used sequentially to ensure optimal imaging of the particles and hence greatest accuracy in reading the codes. The process of reading the code information from the recorded images of particles is as described in GB 2306484 B. It would also be possible to configure the mechanically driven stage such that the vessel is vibrated rapidly. This may be useful to ensure at the start of the scanning process that any particles which rested on their side would topple to the more stable flat orientation required for code reading. In the normal course of events, however, the particles are considered to be static within the vessel such that the images of the particles can be used to not only determine the codes on individual particles but also, in conjunction with a knowledge of the stage motion, the position of each particle can be evaluated. Knowledge of the position of each particle at and after the final code reading process can be of value because it allows the user to either manually of robotically find and extract a particle or particles of choice. Examples of the use of this facility are given below.

Improvements to Combinatorial Processing

The following improvements to the actual methodologies of combinatorial processing are realisable because of the unique ability of the coded silicon particles to allow particle tracking of all particles throughout the split-and-pool process steps by reading codes at each 'split' stage. This is in contrast to conventional 'chemical tagging' of the particles (polymer beads) which allows the process paths of individual beads to be determined only at the termination of the complete compound synthesis process.

In 'conventional' split-and-pool combinatorial processing, bead consumption is not, per se, a major consideration. Accordingly a suitable degeneracy can be selected so as to guarantee (to an acceptable level) that at least one exemplar of every permutation emerges at the end of the synthesis. A typical value of degeneracy is 10, ie: there are ten times as many beads as there are possible permutations of bead paths. In the case of the coded particles described in this invention such a degeneracy represents a cost in silicon and particle processing, and accordingly it is advantageous to reduce this number. A possible strategy is to read the codes on each particle at a point where the collection of particles is split into sub-sets before a given set of parallel reactions, and to seek to identify significantly underrepresented permutations. (These are not the final permutations, but those existing at earlier split stages). Such permutations may be resolved by remixing all, or possible some, of the sub-sets and re-reading the codes. The process results in a significant gain and can be expected to reduce the required degeneracy to some degree.

A second improvement realisable by virtue of the fact that the process paths of all coded particles taking part in the combinatorial synthesis are known is as follows. High Throughput Screening is both resource consumptive and in the case of the polymer resin beads involves difficult manipulation of small objects. A known strategy in this case is that of Pharmacopeia Inc. (Princeton, USA) who have developed a proprietary two stage chemical linker that will allow half of the compound to be cleaved from each bead. By this means sub-sets of 'N' beads of the total 'M' are put in analysis vessels and half the compound from the beads cleaved to result in composite compound solutions. The resulting composite solutions are then checked for activity. If activity is detected then the N beads are removed from

the vessel showing the activity and placed individually into separate vessels (one bead per vessel) whereupon the remaining half of the compound may be cleaved from the bead. These individual compound solutions are then assayed to identify the active compound. By this means the screening of the population of beads involves (M/N)+N assays rather than M, a significant reduction.

Using the coded silicon particles described in this invention we can achieve the same effect by taking two populations of coded particles through identical process steps or by dividing a larger collection of beads into two approximately equal sets at the end of the synthesis process. In either case two distinct sets of beads are extant: A, B, resulting from synthesis with sufficient redundancy to ensure a statistically satisfactory representation of all chemical structures within each set. One set, for instance A, is reserved for later use and the second set B, is divided at random into M subsets of N beads. The exact value of N being determined by statistical considerations relating to the particular synthesis specification. Small variations about N from subset to subset are not detrimental to the methodology as described here. The synthesised compound bound to the individual beads is preferably released by cleaving the linking compound thus releasing the synthesised compounds into the liquid phase (however options to screen with the compound uncleaved are also applicable to this technique) within the analysis vessel containing the N beads. The coded beads are left in-situ within the vessel.

Established screening procedures, advantageously those employed in High Throughout Screening (HTS), are then employed to test for desirable chemical activity in any of the M subsets and hence vessels. Detection of desirable chemical activity by means (for instance) of fluorescence within on of the M vessels indicates that one or more of the N chemical structures it contains may be responsible.

By removing the N coded beads from the vessel it is possible by automated techniques (such as those employed within the main synthesis sequence) or by manual inspection, to determine the codes associated with the particular N beads. Reference to the synthesis database will enable the N structures associated with these codes to be determined. The database may now be interrogated to establish the codes associated with an identical "clone" chemical structure subset within bead set B. This will yield a new list of N codes different from those read from the beads in-situ, but representing a clone subset of N chemical structures. The bead reading system may now be instructed to locate this subset of beads from set B. This identification may be on the basis of beads previously determined spatial coordinates or by direct searching.

Retrieval may be manual, for instance guided by an optical designating spot (for instance a laser) or it may be fully automated by established robotic techniques. The "clone" subset of N beads will then be allocated one per vessel for a second phase of screening with the objective of determining the structure or structures resulting in a positive screen. Reading of the bead code associated with positive screening activity will allow interrogation of the synthesis database and hence elucidation of the chemical structure.

It may be desirable to a skilled chemist to be able to define particular chemical-structural regions of the synthesised library from which beads should be retrieved. Such regions may represent chemical structures with a desired property: for instance they might all share a defined, common, chemical sub-structure (for instance a Benzene ring). Alternatively the chemist may wish to list a set of chemical structures that he wishes to examine. In either case the capacity to extract such particles is predicted by a combinatorial synthesis methodology that offers full deconvolution.

In this invention we can exploit a synthesis database that connects every chemical structure to a unique code thereby enabling the chemist's set or list of desired compounds to be translated to a list of corresponding head codes from which the automated machine vision particle-code reading system can retrieve the appropriate beads. This identification may be on the basis of beads previously determined spatial co-ordinates or by direct searching. Retrieval may be manual, for instance guided by an optical designating spot (for instance a laser) or it may be fully automated by established robotic techniques. An alternative to the specification of a region of space or a list of compounds would be to "sample" chemical space whereby a certain degree of synthesis path variation is specified and only structures exceeding this difference are extracted. By this means a smaller initial screening library may be extracted that indicates broad trends in activity within the library. In either example the retrieved compounds would be cleaved from the supporting bead into liquid phase for subsequent analysis or may be subjected to screening procedures as previously described.

CLAIMS

1. A method for screening to identify a compound of interest from a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available in a database to identify the sequence of reactions experienced by substantially each support particle, the method comprising the steps of:

(a) dividing the compound library into two sets there being a statistically satisfactory representation of all compounds in the library within each set;

(b) sub-dividing a first one of the two sets into a number of sub-sets;

(c) testing for a chemical activity of interest in any one of the said sub-sets and identifying the one or more sub-sets showing the activity of interest and reading the machine readable codes of the support particles in that or those sub-set(s);

(d) checking a database of the tracking data, or data established from the tracking data, to identify machine readable codes of any other support particles which have been through the same steps and therefore, have the same chemical structure of compound on the support particle; and

(e) reading the machine readable codes of the support particles in the second set of particles to locate the one or more support particles having the corresponding machine readable code(s) and retrieving that or those support particles and subjecting it or them to further screening tests to identify the compound of interest.

2. The method as claimed in claim 1 wherein prior to step (b) the compound bound to each support particle is released by relieving the compound linking it to the support particle to release each compound into a liquid medium .

3. The method as claimed in claim 1 or claim 2, wherein in step (c) prior to

reading the machine readable codes of the support particles they are removed from a vessel in which they were tested for chemical activity and transferred to a reading station.

4. The method as claimed in claim 3, wherein the reading of the codes on the coded particles is carried out by firstly placing the particle to be read in a substantially flat, substantially horizontal vessel with a transparent bottom, the area of the bottom being at least double the total area of the particles to be measured; agitating the vessel to form a monolayer of particles on the bottom; and scanning the vessel with an imaging system whilst illuminating the particles.

5. A method as claimed in any preceding claim, wherein the reading station is adapted to automatically read the machine readable codes of the support particles and to automatically identify the one or more support particles with machine readable codes in step (e) that correspond to the codes from the database identified in step (d).

6. The method as claimed in claim 5, wherein the one or more corresponding support particles are automatically retrieved or pinpointed by a laser beam, or other means, from the second set of particles.

7. A system for use in the method of any preceding claim and which comprises at least two primary vessels each containing a respective one of the two sets of support particles, a reading station to read the machine readable codes of the support particles; a database of the machine readable codes and tracking data; and means programmed to identify the machine readable codes of the corresponding

support particles ("clones") to those in the sub set which has been found to have the chemical activity of interest.

8. A system as claimed in claim 7 and further having an automatic retrieval means to pinpoint by a laser beam or other means and/or to robotically retrieve the support particles ("clones") of interest.

9. A method for identifying a group of different but related chemical compounds from a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available in a database to identify the sequence of reactions experienced by substantially each support particle, the method comprising computer searching the database to determine each machine readable code corresponding to a compound structure within the group of compounds of interest.

10. A method as claimed in claim 8, wherein retrieval of the support particles corresponding to the thus-identified machine readable codes is automated either by the relevant particles being high-lighted by designating means such as, a laser or other means and/or fully automatically retrieved by means of robotic automation.

11. A method of building and deconvoluting a combinatorial compound library comprising the steps of:

(a) providing a plurality or set of support particles each with a machine readable code and a data base for tracking data to enable the sequence of synthesis reactions experienced by substantially each support particle to be identified;

(b) suspending the support particles in a fluid;

(c) dividing the fluid containing the particles into a plurality of portions, reading and recording the machine readable codes during or after the division process in order to track the movement of specific particles into respective portions;

(d) subjecting respective portions to specific chemical reactions;

(e) recombining the respective portions; and

(f) repeating steps (c), (d) and (e) as necessary so as to create a compound library in which each member of the library is associated with one or more support particles with a machine readable code and tracking data is available to identify the sequence of reactions experienced by substantially each support particle, and wherein following a division of the particles the codes are read and analysed to determine whether any combinatorial permutation is significantly under represented.

12. A method as claimed in claim 10, wherein any under-representation of any permutation of chemical combination is adjusted for by re-mixing all or some of the sub sets of particles and, optionally, re-reading the machine readable codes.

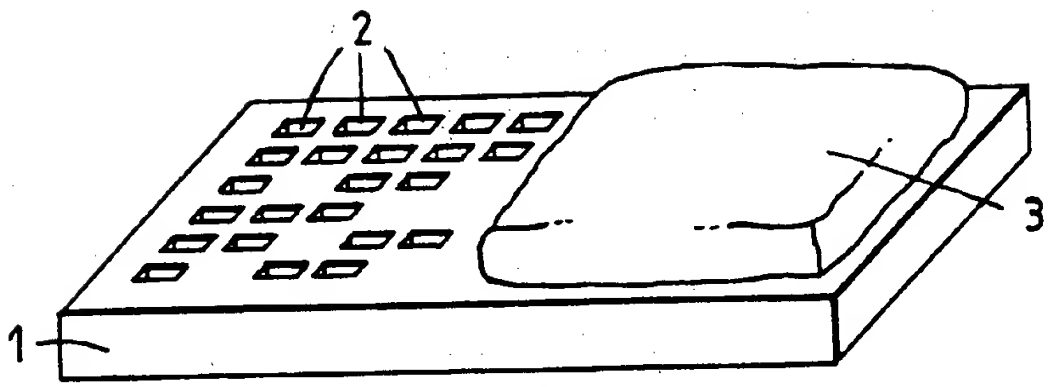


Fig. 1A

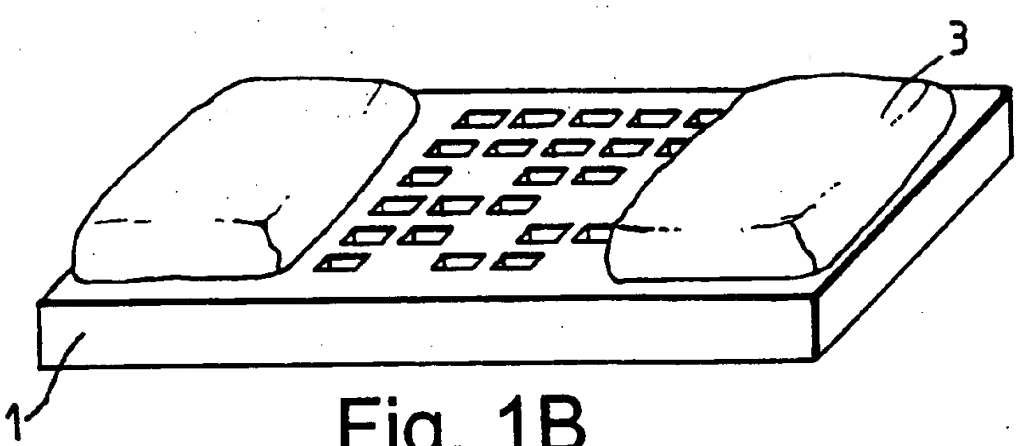
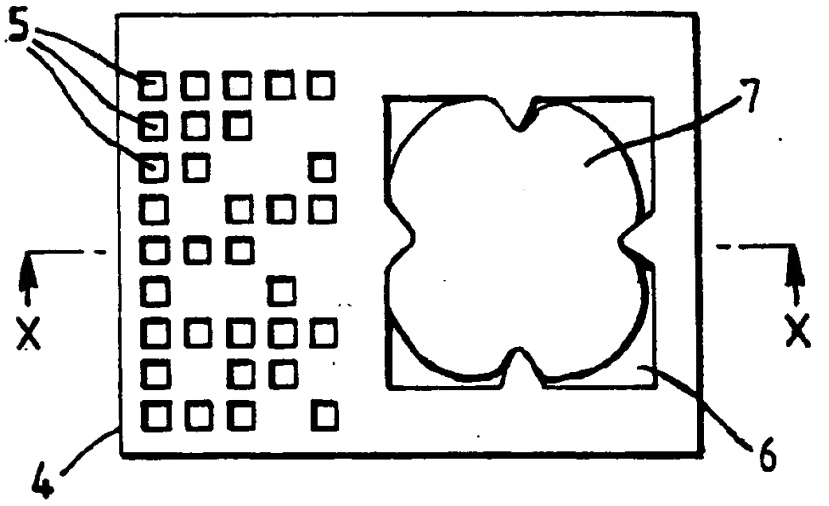


Fig. 1B



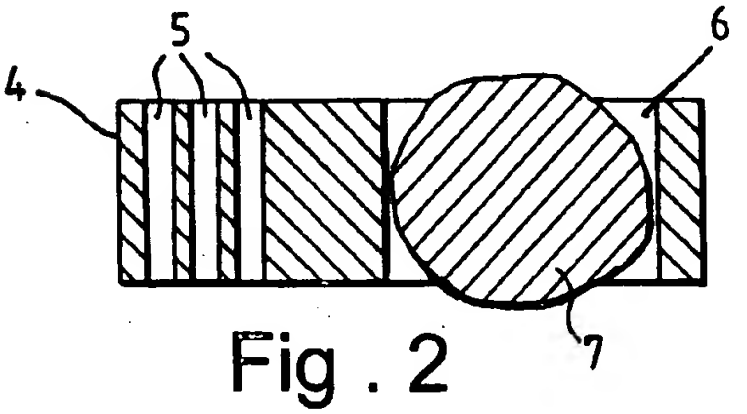


Fig . 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/02108

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01J19/00 G01N33/58 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01J G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	— -/—	1-8, 10-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1999	03/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Stevnsborg, N

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/02108

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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